

FIELD APPLICATION OF BACTERIAL ISOLATE *BACILLUS POLYMYXA* FROM RHIZOSPHERE OF BRINJAL (*SOLANUM MELONGENA L.*)

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ABSTRACT

The present study was conducted with the main purpose to evaluate on field beneficial effect of *Bacillus polymyxa* isolated from rhizosphere of brinjal (*Solanum melongena L.*). Brinjal (*Solanum melongena L.*) cultivar Arka keshav and Arka shirish were used in this study. The isolated bacteria identified to be *Bacillus polymyxa* was used as the inoculant. Inorganic fertilizers such as urea, superphosphate, and muriate of potash were used in reduced doses. The seeds of brinjal were obtained from Division of Vegetable crops. IIHR, Hesaraghatta. Bangalore. The results of field trial revealed that *Bacillus polymyxa* bacterial inoculation significantly increased growth parameters, plant mineral content and yields. The Brinjal (*Solanum melongena L.*) cultivar Arka keshav plants treated with *Bacillus polymyxa* which received full dose of nitrogen and phosphorus showed a 79% increase in root dry weight over uninoculated controls. Plants that received half dose of nitrogen and phosphorus treated with *Bacillus polymyxa* showed a 65% increase in leaf surface area in the first leaf over the uninoculated controls, which received the same level of nitrogen and phosphorus. The plants, which received half dose of nitrogen and phosphorus and treated with *Bacillus polymyxa*, showed a 23% increase in plant nitrogen and 47% increase in plant phosphorus over the uninoculated controls. Plants treated with *Bacillus polymyxa* and received full dose of nitrogen and phosphorus showed a 17% increase in plant nitrogen and 55% increase in plant phosphorus over the uninoculated controls. Plants which received half dose of nitrogen and half dose of phosphorus and treated with *Bacillus polymyxa* showed a 56% increase in copper content over the uninoculated controls. Plants, which received full dose of nitrogen and half dose of phosphorus and treated with *Bacillus polymyxa*, showed 84% increase in plant phosphorus and 94% increase in plant potassium over the uninoculated control. The photosynthetic efficiency of treated plants in all plots was higher than the respective uninoculated controls. The soil phosphorus content during the period of the experiment showed variation. The rhizosphere soil of the treated plots in which brinjal cv. Arka keshav was cultivated showed significantly higher values of available phosphorus content than the control plots. The plots treated with full dose of nitrogen and phosphorus showed a 66% improvement over the uninoculated controls. The rhizosphere soil of the treated plots in which Arka shirish was cultivated had significantly higher available phosphorus and nitrogen content in the soil than the control plots. In conclusion, the bacterial isolate from brinjal (*Solanum melongena L.*) plants identified as *Bacillus polymyxa* when used as inoculum in field trials showed significant improvement in growth parameters of treated plants over the uninoculated controls at all levels of fertilizer application.

KEYWORDS: Brinjal Bacterial Isolate, Field Trial, *Solanum Melongena L.*, *Bacillus Polymyxa*, *Azospirillum*, Arka Keshav, Arka Shirish

Received: Oct 21, 2021; Accepted: Nov 11, 2021; Published: Nov 17, 2021; Paper Id.: IJMPDEC202114

INTRODUCTION

Beneficial associative microorganisms isolated from many crop plants have been effectively used to fertilize the same crops. (Subba Rao, 1983) Many such application studies are related to cereals, legumes and vegetables. Barea and Brown (1974) reported that inoculation with *Azotobacter paspali* significantly improved the growth of tomato and lettuce. Plant root association with *Azospirillum* has been reported to improve plant productivity. (Okon et al., 1977; Cohen et al., 1980, Eid et al., 1984; Jadhav et al., 1986) Alice and Subramanian, 1987; Polsinelliet al., 1988; Tilakand Dwivedi, 1990; and Smith 1984). Legumes that responded well to this type of fertilizer treatment were winged bean, soybean, cow pea (Quimio and Cadapan, 1988) and Peas. (El Mokadem and Badawi, 1992) Vegetables such as Yam, sweet potatoes, beet root, potatoes, tomato, sugar beet and cabbage showed significant improvement when treated with associative bacteria isolated from the stem of the same crop (Crossman and Hill, 1987; Hassouna et al., 1991; Hegazi et al., 1983; Hill et al., 1983; Julca-de! 1991, Mineev et al., 1991, Martin et al., 1992 and Pati and Chandra, 1993).

Other crops which recorded significant improvement when given this treatment were mustard, sugarcane, Kallar grass, green house bell pepper, lupine, lodge pine, banana, jute, rye and coconut palm. (Saha et al., 1985; Reinhold, 1986; George 1990; Bumgardner and Mardon, 1992; El Mokadem and Badawi, 1992, and Pati, 1992; Pati and Chandra, 1993) Cereals such as rice, wheat, pearl millet, oats, zea mays, corn, Barley and sorghum (Badaway et al., 1974; Baldani et al., 1983a and b; Subba Rao, 1983; Purushothaman and obilisemi, 1984; Prasad and Singh 1987, Bouton 1988; Quimio and Cadapan; 1988; Gallo et al., 1989) showed significant improvement in plant biomass and grain yield after inoculation with *Azospirillum*. Different levels of fertilizers were applied along with the isolated associative microorganisms to supplement biofertilization. Settha-siripin (1987) studied the effect of using 14 species of associative nitrogen fixing bacteria on sugarcane in an inoculation experiment using four levels of nitrogen. He reported significant increase in plant biomass, total plant nitrogen in treated plants over that of uninoculated controls. Murali and Purushothaman, (1988), isolated a number of efficient nitrogen fixing bacteria identified to be *Azospirillum* from the roots of upland rice. These were successfully used as biofertilizer in a field experiment using different fertilizer levels. The treated rice plants showed significant increase in grain and straw yield. Grain yield was highest in *Azospirillum* treated plants, which received 75-Kg hectare- 1nitro gen than the plants, which received 100 kg fertilizer N/hectare. Gopalaswamy and Vidyasekaran (1987, 1988) studied the response of *Azospirillum* inoculation on different varieties of rice using different levels of fertilization. They reported that treated plants produced higher number of productive tillers, and had increased straw and grain yield. The split application of the inoculum treatment (seed, seedling and soil inoculation) gave the highest results in the above experiment. Rajagopalan and Rangaswamy, (1988) studied the effect of *Azospirillum* inoculation on rice yield in both Kharif and Rabi season. They used farm yard manure (FYM) mixed with *Azospirillum* and different levels of nitrogen in their experiment. They found significant yield improvement during the rabi season.

Furthermore, Bashan, 1986, Bashan, et al., (1990) reported that enhanced growth of wheat and soybean plants inoculated with *Azospirillum brasiliense* was not necessarily due to enhanced mineral uptake. They did not report any other factor responsible for the significant increase in yield and other parameters in treated plants. The germination counts in the treated beds were significantly higher than in uninoculated controls. Balasubramanian, (1987) observed enhanced tillering by combining fertilizer nitrogen at different levels along with *Azospirillum* inoculation. They reported significant increase in grain and straw yield at all levels of nitrogen application. Plants treated with *Azospirillum* and half dose of nitrogen showed maximum increase in plant dry weight. Treated plants which received full doze of nitrogen showed

significant increase in tiller numbers, plant dry weight and grain yield. Treated plants, which received lower levels of fertilization showed pronounced response than the uninoculated controls. The mean grain yield at 50% nitrogen fertilizer with Azospirillum treatment equally yield with 100% nitrogen fertilizer. Significant increase was recorded at all levels.

With this scenario, the present study field trial was conducted to evaluate the potential of *Bacillus polymyxa* isolated from brinjal as a biofertilizer. The natural occurrence, distribution, survival of *Bacillus polymyxa* after seed inoculation, along with different levels of inorganic fertilizers were done to evaluate nitrogen and phosphorus use efficiency in Kharif and Rabi seasons.

MATERIALS AND METHODS

Brinjal (*Solanum melongena L.*) cultivar *Arka keshav* and *Arka shirish* were used in this study. The isolated bacteria identified to be *Bacillus polymyxa* was used as the inoculant. Inorganic fertilizers such as urea, superphosphate, and muriate of potash were used in reduced doses. The seeds of brinjal were obtained from Division of Vegetable crops. IIHR, Hesaraghatta. Bangalore.

Brinjal (*Solanum melongena L.*) cv *Arka keshav* seedlings were raised in nursery beds measuring 1.0m x 1.5m. One of the nursery beds received 500ml of 48hr broth of *Bacillus polymyxa* mixed with FYM. The other nursery bed received 250ml of 48-hour culture of *Bacillus polymyxa*, and 250 ml of 48hr culture of Azospirillum. The bacterial inoculants were remixed with FYM and applied. The third bed served as the control, which received 500 mL of heat, killed bacterial culture broth. Seeds of Brinjal (*Solanum melongena L.*) were sown in rows 5 cms apart above the inoculum in the field soil at a depth of 2 cms. Starter cultures of *Bacillus polymyxa* were subcultured in Burk's media (Subba Rao 1983) and multiplied using TYMB Media (pH 7.0). (Dalton, 1980)

One month old seedlings were transplanted to the experimental plots measuring 2m x 2m in rows with a spacing of 80 cms between rows and 40 cms between plants. The soil had a pH of 6.3 having an organic nitrogen content of 3.03 ppm, 0.04 ppm phosphorus and 0.67% of organic carbon. All the seedlings were given a drenching of the respective inoculum before transplantation. The plots were topped with FYM. Recommended fertilizer dosage for Brinjal i.e., 170N: SOP: 60K was applied in the form of urea, superphosphates and muriate of potash respectively. Nitrogen and phosphorus were applied in different levels viz., NrPf. NrPH, NHPf. and NHPH ('f' means full dosage and 'h' means half dosage). Each level had treatment plots and control plots in triplicates. Biometric observations and physiological parameters recorded were shoot fresh weight, plant height, photosynthetic efficiency, leaf surface area, mineral content and yield. Nitrogen and phosphorus content of the soil were analyzed periodically. The average maximum and minimum temperature during the period of the experiment was 30°C and 14°C respectively. The average relative humidity was 58.30% and the average rainfall was 46.3 mm.

Photosynthetic efficiency was measured in percentage using photosynthetic efficiency analyzer (Hansa Tek) which measures the Dark reaction of photosynthesis. The leaf area was measured using a Skyla leaf area analyzer. Measurements were made in the fully expanded intact leaf developed at the 3rd internode. The leaf area was expressed in cm². The yield was collected periodically and the data was expressed in kg/plot.

To determine the plant nitrogen, Kjeldhal procedure (Jackson 1973) was followed. Leaf samples were collected from the field and air dried in a hot air oven at 60°C. The air dried samples were ground and sieved. 100 mg of the dried plant material was digested in Kjeldhal digestion flasks with 5ml of concentrated sulphuric acid. Digestion tablets were

added to aid the digestion. 1% v/v hydrogen peroxide was added at the end of the digestion. The volume in the cooled digested flasks was made upto 100mL. 25 mL of the diluted digest along with 15mL of 40% sodium hydroxide was distilled. The distillate was titrated against 0.01N hydrochloric acid.

Plant phosphorus, potassium and other microelements were determined by the method described by Jackson (1973). 100 mg of the plant material, which was dried, ground and sieved and digested using 10 ml of diacid mixture. The volume was made upto 100 ml and filtered and the plant mineral content was analyzed using an atomic spectrophotometer (Perkin Elmen 5000 Atomic absorption spectrometer). The amount of plant potassium was analysed using a flame photometer. The phosphorus, calcium, magnesium, sulphur and potassium content were expressed in percentage. The amount of the other elements was expressed in parts per million.

Nitrogen content of the soil was analysed by the total carbon method of Walkley and Black described by Jackson (1973). One gram of finely powdered and sieved soil was taken in a 500ml flask. 10ml of 1N potassium dichromate and 20ml of concentrated sulphuric acid was added and the flask was placed in a shaker for 30 minutes for digestion. The digest was made up to 200ml and left to stand for 30 minutes. The excess chromic acid left over un-reduced in the digest was determined by titration with standard ferrous sulphate using diphenylamine as an indicator. The appearance of apple green colour indicated the end of the reaction. The amount of nitrogen in the soil was indirectly calculated by the amount of total organic compound present in the soil.

The soil phosphorus content was estimated by the Bray method (Jackson, 1973). Ammonium molybdate and stannous chloride were added to the soil filtrate and the developed colour was read at 690nm. The phosphorus content was calculated using a standard graph. Dilutions of potassium hydrogen phosphate in the range of 0.0 to 2.0 mg/L was used to prepare the standard graph with OD on the X axis and concentration on the Y axis.

Nitrogenase activity in rhizoplane and phylloplane was detected by the acetylene reduction assay following the method of Turner and Gibson (1980). Two months old brinjal plants (treated and control) were taken from the field. The roots and leaves were separated and washed in running water followed by washing with sterile distilled water and cut into 1 inch bits. The Nitrogenase activity in these plant parts was estimated by acetylene reduction technique using a gas chromatograph (Hewlett Packard series 5000). The time period between the harvest of the plants and Nitrogenase assay was 25 mins. The plant materials were kept in 250mL flasks, which was made airtight. 1mL of 10% acetylene was injected into the flask after drawing out the required volume of air. 1mL of the gas phase was analysed after 1 hr of incubation at 37°C.

Field experiments were conducted to test the response of brinjal to inoculation with *Bacillus polymyxa*. Brinjal (*Solanum melongena L.*) cvs Arka Keshav and Arka Shirish were treated with *Bacillus polymyxa* during the Kharif season. Interaction of *Bacillus polymyxa* with Azospirillum was also tested.

RESULTS

Results of the field trial revealed that bacterial inoculation significantly increased growth parameters plant mineral content and yields. (Plate 1a, 1b, 2a, and 2b) The Brinjal (*Solanum melongena L.*) cultivar Arka keshav plants treated with *Bacillus polymyxa* which received full dose of nitrogen and phosphorus showed a 79% increase in root dry weight over uninoculated controls. Plants treated with Azospirillum which received half dose of nitrogen and Phosphorus showed a 49% increase in shoot dry weight over the uninoculated control. Plants treated with *Bacillus polymyxa* showed a 90%

increase in plant height and 94% increase in photosynthetic efficiency and 83% increase in yield over the uninoculated control in treatment plots that received half dose of nitrogen and phosphorus. (Table-1) Plots that received full dose of nitrogen and phosphorus showed a 65% increase in plant height and 69% increase in yield over the uninoculated controls, which received the same level of nitrogen and phosphorus. (Table 1-3) Plants that received half dose of nitrogen and phosphorus treated with *Bacilluspolymyxa* showed a 65% increase in leaf surface area in the first leaf over the uninoculated controls, which received the same level of nitrogen and phosphorus. (Table-4& 5)



Plate 1a: 30 day old Brinjal (*Solanum melongena L.*) cv. Arka keshav cultivated using *Bacillus polymyxa*



Plate 1b: Control Plants

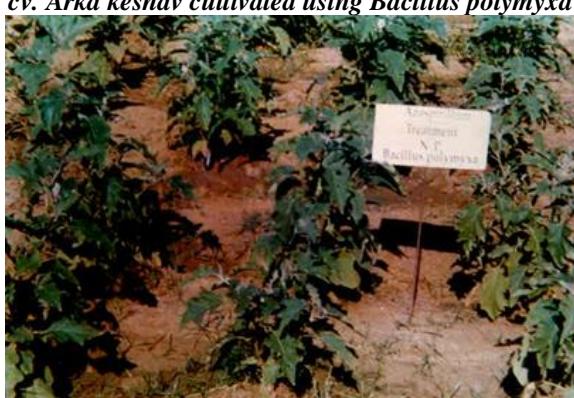


Plate 2a: 90 day old Brinjal (*Solanum melongena L.*) cv. Arka keshav cultivated using *Bacillus polymyxa* at half levels of nitrogen and phosphorous fertilizer at IIHR, Hessaraghatta



Plate 2b: Control plants cultivated at the same level (NhpH)

Figure 1

Table 1: Growth Parameters of Brinjal (*Solanum Melongena L.*) CV. Arka Keshav Plants Fertilized with *Bacillus Polymyxa* and *Azospirillum* in a Filed Trial using Four Fertilizer Levels

| Treatment | | Shoot fresh weight (kg) | % Variation over control | Plant height (cms) | % Variation over control | (3/4) Photosynthetic efficiency | % Variation over control | Yield (kg/plot) | % Variation over control |
|-------------------|-------|-------------------------|--------------------------|--------------------|--------------------------|---------------------------------|--------------------------|-----------------|--------------------------|
| Control | Nr Pr | 1.593 | | 66.43 | | 0.693 | | 4.250 | |
| Control | Nh Pr | 1.310 | | 54.58 | | 0.478 | | 3.750 | |
| Control | Nr Pb | 1.575 | | 64.58 | | 0.502 | | 4.720 | |
| Control | Nh Ph | 1.132 | | 45.39 | | 0.412 | | 3.100 | |
| <i>Bp</i> treated | Nr Pr | 1.937 | 21 | 96.41 | 45 | 0.989 | 42 | 8.500 | 100 |
| <i>Bp</i> treated | Nh Pr | 1.712 | 30 | 86.76 | 58 | 0.703 | 47 | 7.000 | 86 |

Table 1: Contd.,

| | | | | | | | | | |
|-------------------|-------|-------|--------|-------|----|--------------|----|-------|----|
| <i>Bp</i> treated | Nr Ph | 1.833 | 30 | 87.89 | 36 | 0.785 | 56 | 8.250 | 74 |
| <i>Bp</i> treated | Nh Ph | 1.446 | 27 | 74.8 | 51 | 0.642 | 55 | 5.800 | 87 |
| Azo treated | Nr Pr | 1.837 | 15 | 79.29 | 19 | 0.801 | 15 | 7.900 | 85 |
| Azo treated | Nh Pr | 1.562 | 19 | 60.66 | 11 | 0.621 | 29 | 6.935 | 84 |
| Azo treated | Nr Ph | 1.890 | 20 | 78.81 | 22 | 0.701 | 39 | 7.500 | 58 |
| Azo treated | Nh Ph | 1.286 | 13 | 49.34 | 08 | 0.621 | 50 | 4.760 | 53 |
| "F" test | | ** | | ** | | ** | | | |
| CD at 5% | | 0.05 | 9.124 | 0.379 | | 2.778 | | | |
| SEM | | 2.388 | 26.757 | 3.027 | | 1.115 | | | |

*-Significant, **-highly significant, *Bp* - *Bacillus polymyxa*, Azo. - Azospirillum, Nr Pr – Full dose of nitrogen and phosphorus, Nb Pr - half dose of nitrogen and full dose of phosphorus, Nr P – Full dose of nitrogen and half dose of phosphorus, N Pb – half dose of nitrogen and phosphorus, Fertilizer dosages as recommended for *Solanum melongena* L. keshav plants

Table 2: Growth Parameters of Brinjal (*Solanum Melongena* L.) CV. Arka Keshav Plants Fertilized with Bacterial Inoculants in a Field Trial at Three Fertilizer Levels

| Treatment | Shoot fresh weight (kg) | % Variation | Shoot dry weight (gm) | % Variation | Root dry weight (gm) | % Variation |
|---|-------------------------------|-------------|-----------------------|-------------|----------------------|-------------|
| Control N ₀ P ₀ | 0.721 | | 52.33 | | 3.86 | |
| Control NhP | 0.864 | | 78.33 | | 14.61 | |
| Control NrPh | 1.650 | | 153.33 | | 20.83 | |
| <i>Bp</i> treated N ₀ P ₀ | 0.900 | 24 | 78.113 | 39 | 6.13 | 58 |
| <i>Bp</i> treated NP | 1.308 | 51 | 124.66 | 59 | 22.81 | 56 |
| <i>Bp</i> treated NP | 1.952 | 18 | 167.66 | 09 | 27.43 | 31 |
| <i>Bp</i> & Azo Treated | N ₀ P ₀ | 26 | 80.66 | 54 | 6.76 | 75 |
| <i>Bp</i> & Azo Treated | Nh Ph | 40 | 118.33 | 51 | 19.36 | 32 |
| <i>Bp</i> & Azo Treated | N P | 02 | 193.66 | 26 | 23.76 | 14 |
| 'F' test | * | | | | * | |
| CD at 5% | 0.09 | | 0.06 | | 3.16 | |
| SEM | 1.351 | | 50.591 | | 1.077 | |

*-Significant, **-highly significant, *Bp* - *Bacillus polymyxa*, Azo - Azospirillum, N₀P₀ – Zero doze of nitrogen and phosphorus, NP – Full dose of nitrogen and phosphorus, Nh P – Half dose of nitrogen and phosphorus, N P – Full dose of nitrogen and phosphorus, Fertilizer dosages as recommended for *Solanum melongena* L. cv. Arka keshav plants

Table 3: Growth Parameters of Brinjal (*Solanum Melongena* L.) cv. Arka Keshav Plants Fertilized with Bacterial Inoculants in a Field Trial at Three Fertilizer Levels

| Treatment | Plant Height (cms) | % Variation | % Photosynthetic Efficiency | % Variation Over Control | Yield (kg/plot) | % Variation Over Control |
|---|--------------------|-------------|-----------------------------|--------------------------|-----------------|--------------------------|
| Control N ₀ P ₀ | 23.46 | | 0.153 | | 3.667 | |
| Control Nh P | 34.26 | | 0.303 | | 3.686 | |
| Control NrPh | 40.63 | | 0.360 | | 5.303 | |
| <i>Bp</i> treated N ₀ P ₀ | 35.83 | 52 | 0.271 | 77 | 5.953 | 62 |
| <i>Bp</i> treated N Ph | 50.83 | 48 | 0.570 | 88 | 7.300 | 98 |
| <i>Bp</i> treated N P | 71.63 | 76 | 0.563 | 56 | 9.698 | 82 |

Table 3: Contd.,

| | | | | | | | |
|-------------------------|-------------------------------|-------|----|-------|----|-------|----|
| <i>Bp</i> & Azo treated | N ₀ P ₀ | 33.73 | 46 | 0.266 | 73 | 4.860 | 82 |
| <i>Bp</i> & Azo treated | NP | 52.90 | 54 | 0.476 | 57 | 6.266 | 69 |
| <i>Bp</i> & Azo treated | N _r P | 69.50 | 71 | 0.596 | 65 | 8.900 | 67 |
| “F” test | * | | | * | | * | |
| CD at 5% | 6.19 | | | 0.708 | | 1.878 | |
| SEM | 5.529 | | | 3.021 | | 1.141 | |

*-Significant, **-highly significant, *Bp* - *Bacillus polymyxa*, Azo - Azospirillum, N₀P₀- Zero dose of nitrogen and phosphorus, N_hP – Half dose of nitrogen and phosphorus, N Ph- Full dose of nitrogen and half dose of phosphorus, NP – Full dose of nitrogen and phosphorus, Fertilizer dosages as recommended for *Solanum melongena L.* cv. Arka keshav plants.

Table 4: Leaf Surface Area of Brinjal (*Solanum Melongena L.*) Cv. Arka Keshavplants Cultivated using Four Fertilizer Levels and Bacterial Inoculants

| Treatment | First leaf cm ² | % variation over control | Second leaf cm ² | % variation over control | Third leaf cm ² | % variation over control | Fourth leaf cm ² | % variation over control |
|---|-------------------------------|-----------------------------|--------------------------------|-----------------------------|-------------------------------|-----------------------------|--------------------------------|-----------------------------|
| Control N _r P _r | 60.483 | | 62.58 | | 53.503 | | 32.196 | |
| Control N _h P _r | 46.150 | | 44.160 | | 45.466 | | 25.523 | |
| Control N _r P _h | 55.263 | | 52.426 | | 53.523 | | 29.270 | |
| Control N _h P _h | 55.700 | | 42.246 | | 24.863 | | 21.660 | |
| <i>B. polymyxa</i> treated N _r P _r | 91.166 | 49 | 108.870 | 73 | 101.200 | 90 | 63.170 | 96 |
| <i>B. polymyxa</i> treated N _h P _r | 89.863 | 94 | 73.440 | 66 | 82.070 | 80 | 46.120 | 80 |
| <i>B. polymyxa</i> treated N _r P _h | 90.230 | 49 | 95.269 | 81 | 102.780 | 92 | 58.766 | 02 |
| <i>B. polymyxa</i> treated N _h P _h | 91.780 | 65 | 65.490 | 55 | 49.740 | 76 | 35.263 | 62 |
| <i>Azospirillum</i> treated N _r P _r | 91.130 | 49 | 68.433 | 09 | 103.230 | 92 | 56.806 | 76 |
| <i>Azospirillum</i> treated N _h P _r | 75.480 | 63 | 63.213 | 43 | 72.913 | 60 | 45.876 | 79 |
| <i>Azospirillum</i> treated N _r P _h | 94.230 | 70 | 87.283 | 66 | 88.140 | 64 | 50.250 | 71 |
| <i>Azospirillum</i> treated N _h P _h | 79.530 | 74 | 67.870 | 60 | 43.890 | 100 | 41.573 | 91 |
| F ² test | * | | * | * | * | | * | * |
| CD at 5% | 06.289 | | 35.578 | | 26.540 | | 9.484 | |
| SEM | 2.144 | | 6.722 | | 2.231 | | 3.234 | |

* significant

** highly significant

N_rP_r Full doze of nitrogen and phosphorus*B. polymyxa* *Bacillus polymyxa*N_hP_r Half doze of nitrogen and full doze of phosphorusN_rP_h full doze of nitrogen and half doze of phosphorusFertilizer dosages as recommended for *solanum melongena L.* plantsN_hP_h half doze of nitrogen and phosphorusTable 5: Leaf Surface Area of Brinjal (*Solanum Melongena L.*) CV. Arka Keshav Plants Fertilized with Three Fertilizer Levels and Bacterial Inoculants in a Field Trial

| Treatment | | First leaf cm ² | % Variation over control | Second leaf cm ² | % Variation over control | Third leaf cm ² | % Variation over control | Fourth leaf cm ² | % Variation over control |
|------------------|-------------------------------|----------------------------|--------------------------|-----------------------------|--------------------------|----------------------------|--------------------------|-----------------------------|--------------------------|
| Control | N ₀ P ₀ | 46.22 | | 57.8 | | 39.86 | | 28 | |
| Control | N _h Ph | 45.42 | | 50.06 | | 44.28 | | 41.91 | |
| Control | N _r P _r | 54.54 | | 57.66 | | 53.6 | | 45.32 | |
| Bp treated | N ₀ P ₀ | 71.66 | 55 | 77.10 | 33 | 67.44 | 69 | 45.68 | 63 |
| Bp treated | N _h Ph | 65.92 | 45 | 85.25 | 64 | 73.87 | 70 | 57.82 | 37 |
| Bp treated | N _r P _r | 73.38 | 33 | 75.90 | 31 | 85.36 | 59 | 77.64 | 71 |
| Bp & Azo treated | N ₀ P ₀ | 73.82 | 59 | 67.56 | 16 | 53.87 | 35 | 43.78 | 56 |
| Bp & Azo treated | N _h P | 88.98 | 95 | 87.08 | 73 | 72.51 | 67 | 46.58 | 11 |
| Bp & Azo treated | N _r P _r | 64.86 | 18 | 114.76 | 99 | 82.94 | 54 | 70.46 | 54 |

Table 5: Contd.,

| | | | | | | | | | |
|-------------|-------------------------------|--------|----|--------|----|-------|----|-------|----|
| Azo treated | N ₀ P ₀ | 50.66 | 08 | 64.00 | 10 | 51.1 | 28 | 44.79 | 59 |
| Azo treated | NPb | 62..21 | 36 | 74.34 | 48 | 68.86 | 59 | 50.86 | 21 |
| Azo treated | NrPr | 56.65 | 03 | 87.66 | 52 | 74.32 | 38 | 56.56 | 24 |
| | “F” test | * | * | | * | | | * | |
| CD at 5% | | 45.94 | | 23.77 | | 16.13 | | 31.16 | |
| SEM | | 9.79 | | 144.44 | | 9.31 | | 8.13 | |

*-Significant, **-highly significant, Bp - *Bacillus polymyxa*, Azo - Azospirillum, N₀P₀ - Zero dose of nitrogen and phosphorus, NrPr - full dose of nitrogen and phosphorus, NPb, half dose of nitrogen and phosphorus, Fertilizer doses as recommended for *Solanum melongena* L. cv. Arka keshav plants

The plants, which received half dose of nitrogen and phosphorus and treated with *Bacillus polymyxa*, showed a 23% increase in plant nitrogen and 47% increase in plant phosphorus over the uninoculated controls. Plants treated with *Bacillus polymyxa* and received full dose of nitrogen and phosphorus showed a 17% increase in plant nitrogen and 55% increase in plant phosphorus over the uninoculated controls. Plants which received half dose of nitrogen and half dose of phosphorus and treated with *Bacillus polymyxa* showed a 56% increase in copper content over the uninoculated controls (Table-6 & 7). Plants, which received full dose of nitrogen and half dose of phosphorus and treated with *Bacillus polymyxa*, showed 84% increase in plant phosphorus and 94% increase in plant potassium over the uninoculated control.

Table 6: Mineral Content of Brinjal (*Solanum Melongena* L.) CV. Arka Keshav Plants Fertilized with Bacterial Inoculants and Inorganic Fertilizer in a Field Trial

| Treatment | | Copper (ppm) | % Variation over control | % Calcium | % Variation over control | % Magnesium | % Variation over control | % Sulphur | % Variation over control | Manganese (ppm) | % Variation over control |
|------------------|------|--------------|--------------------------|-----------|--------------------------|-------------|--------------------------|-----------|--------------------------|-----------------|--------------------------|
| Control | NrPr | 0.057 | | 0.069 | | 0.077 | | 0.070 | | 0.153 | |
| Control | NhPr | 0.041 | | 0.059 | | 0.059 | | 0.037 | | 0.076 | |
| Control | NrPh | 0.051 | | 0.067 | | 0.067 | | 0.061 | | 0.127 | |
| Control | NhPh | 0.039 | | 0.061 | | 0.051 | | 0.036 | | 0.068 | |
| Bp treated | NrPr | 0.097 | 70 | 0.121 | 54 | 0.091 | 90 | 0.087 | 24 | 0.227 | 48 |
| Bp treated | NhPh | 0.069 | 69 | 0.079 | 14 | 0.076 | 28 | 0.049 | 32 | 0.137 | 44 |
| Bp treated | NrPh | 0.071 | 39 | 0.101 | 46 | 0.081 | 20 | 0.069 | 13 | 0.201 | 58 |
| Bp treated | NhPh | 0.061 | 56 | 0.108 | 56 | 0.071 | 39 | 0.046 | 27 | 0.122 | 79 |
| Bp & Azo treated | NrPr | 0.081 | 42 | 0.089 | 28 | 0.089 | 15 | 0.081 | 15 | 0.194 | 26 |
| Bp & Azo treated | NbPr | 0.073 | 78 | 0.071 | 20 | 0.066 | 11 | 0.041 | 10 | 0.097 | 27 |
| Bp & Azo treated | NrPb | 0.061 | 19 | 0.081 | 20 | 0.079 | 17 | 0.067 | 09 | 0.197 | 55 |
| Bp & Azo treated | NhPb | 0.053 | 74 | 0.053 | 31 | 0.057 | 11 | 0.039 | 08 | 0.126 | 85 |
| ‘F’ Test | | * | | * | | * | | * | | * | |
| CD at 5% | | 0.121 | | 0.062 | | 0.052 | | 0.042 | | 0.121 | |
| SEM | | 0.167 | | 3.681 | | 0.194 | | 0.149 | | 0.167 | |

*-Significant, **-highly significant, Bp - *Bacillus polymyxa*, Azo - Azospirillum, NrPr - Full dose of nitrogen and phosphorus, NPr - Half dose of nitrogen and full dose of phosphorus, NP - Full dose of nitrogen and half dose of phosphorus, NhPh, half dose of nitrogen and phosphorus, Fertilizer doses as recommended for *Solanum melongena* L. plants

Table 7: Mineral Content of Brinjal (*Solanum Melongena L.*) CV. Arka Keshav Plants Cultivated with using four Fertilizer Levels and Bacterial Inoculants in a Field Trial

| Treatment | | Nitrogen (ppm) | 3/4 Variation over control | 3/4 Phosphorus | 3/4 Variation over control | 3/4 Potassium | 3/4 Variation over control | Iron (ppm) | 3/4 Variation over control | Zinc (ppm) | 3/4 Variation over control |
|-------------------------|------|----------------|----------------------------|----------------|----------------------------|---------------|----------------------------|------------|----------------------------|------------|----------------------------|
| Control | NrPr | 16.361 | | 0.596 | | 0.401 | | 12.209 | | 0.472 | |
| Control | NhPr | 7.466 | | 0.331 | | 0.227 | | 10.108 | | 0.364 | |
| Control | NrPh | 8.633 | | 0.483 | | 0.201 | | 11.306 | | 0.426 | |
| Control | NhPh | 7.563 | | 0.401 | | 0.667 | | 9.841 | | 0.341 | |
| <i>Bp</i> treated | NrPr | 19.171 | 17 | 0.986 | 55 | 0.357 | 66 | 23.104 | 89 | 0.683 | 44 |
| <i>Bp</i> treated | NhPh | 10.172 | 36 | 0.583 | 76 | 0.589 | 57 | 18.313 | 81 | 0.563 | 54 |
| <i>Bp</i> treated | NrPh | 12.834 | 48 | 0.896 | 84 | 0.081 | 94 | 17.636 | 55 | 0.621 | 45 |
| <i>Bp</i> treated | NhPh | 9.364 | 23 | 0.591 | 47 | 0.313 | 55 | 13.998 | 42 | 0.541 | 36 |
| <i>Bp</i> & Azo treated | NrPr | 17.508 | 07 | 0.989 | 65 | 0.686 | 28 | 20.812 | 70 | 0.583 | 23 |
| <i>Bp</i> & Azo treated | NhPr | 8.346 | 11 | 0.521 | 57 | 0.316 | 39 | 17.614 | 74 | 0.486 | 33 |
| <i>Bp</i> & Azo treated | NrPh | 9.784 | 13 | 0.826 | 71 | 0.518 | 70 | 16.296 | 44 | 0.537 | 26 |
| <i>Bp</i> & Azo treated | NhPh | 8.782 | 16 | 0.521 | 29 | 0.296 | 47 | 12.213 | 24 | 0.421 | 23 |
| p-value | | ** | | * | | * | | * | | * | |
| CD at 5% | | 0.492 | | 0.457 | | 0.256 | | 0.543 | | 0.259 | |
| SEM | | 0.164 | | 0.159 | | 0.132 | | 0.381 | | 0.078 | |

*-Significant, **-highly significant, *Bp* - *Bacillus polymyxa*, Azo - Azospirillum, NrPr – Full dose of nitrogen and phosphorus, NbPr – Half dose of nitrogen and full dose of phosphorus, NrPb – Full dose of nitrogen and half dose of phosphorus, NhP, half dose of nitrogen and phosphorus, Fertilizer dosages as recommended for *Solanum melongena L.* Plants

Inoculation of brinjal plants cv. *Arka shirish* with *Bacillus polymyxa* (BBI) supplemented with half level of nitrogen and phosphorus increased shoot fresh weight by 30% over the uninoculated controls which received the same level of nitrogen and phosphorus. Plants treated with *Bacillus polymyxa* which received half dose of nitrogen and phosphorus showed 27% increase in shoot fresh weight and a 51% increase in plant height and 55% increase in photosynthetic efficiency and 87% improvement in yield over the uninoculated control which received the same level of fertilizers. Plants treated with Azospirillum, which received half dose of Phosphorus and nitrogen, showed 50% increase in photosynthetic efficiency over the uninoculated control (Table-8, 2&3). The photosynthetic efficiency of treated plants in all plots was higher than the respective uninoculated controls.

Table 8: Growth Parameters of Brinjal (*Solanum Melongena L.*) CV. Arka Keshav Plants Fertilized with *Bacillus Polymyxa* and *Azospirillum* in a Filed Trial using Four Fertilizer Levels

| Treatment | | Plant height (cms) | % Improvement over control | % Photosynthetic efficiency | % Improvement over control | Yield (kg/plot) | % Improvement over control |
|-------------------|-------------------|--------------------|----------------------------|-----------------------------|----------------------------|-----------------|----------------------------|
| Control | NrPr | 59.45 | | 0.54 | | 4.33 | |
| Control | NhPr | 48.35 | | 0.46 | | 3.20 | |
| Control | NrPh | 49.67 | | 0.45 | | 4.33 | |
| Control | NhPh | 36.95 | | 0.38 | | 2.36 | |
| <i>Bp</i> treated | N _H Pr | 98.57 | 65 | 0.96 | 77 | 7.36 | 69 |
| <i>Bp</i> treated | N _h Pr | 80.36 | 66 | 0.68 | 47 | 5.66 | 76 |
| <i>Bp</i> treated | NhP | 87.75 | 76 | 0.78 | 73 | 7.33 | 69 |
| <i>Bp</i> treated | NPh | 74.08 | 90 | 0.74 | 94 | 5.04 | 83 |
| Azo treated | NP _h | 87.90 | 47 | 0.80 | 48 | 7.93 | 85 |
| Azo treated | NrPh | 71.48 | 47 | 0.78 | 69 | 5.11 | 59 |
| Azo treated | N _h Pr | 80.50 | 62 | 0.75 | 66 | 6.67 | 56 |
| Azo treated | Nh | 49.88 | 34 | 0.65 | 71 | 6.76 | 56 |
| p Test | | * | | * | | * | |
| CD at 5% | | 0.05 | | 0.07 | | 0.06 | |
| SEM | | 51.40 | | 0.49 | | 5.64 | |

*-Significant, **-highly significant, *Bp* - *Bacillus polymyxa*, Azo - *Azospirillum*, NrPr – full dose of nitrogen and phosphorus, NrPh – full dose of nitrogen and half dose of phosphorus, NhPf – half dose of nitrogen and full dose of phosphorus, NhPh – half dose of nitrogen and phosphorus, Fertilizer doses as recommended for *Solanum melongena L.* cv. *Arka keshav* plants

DISCUSSION

The bacteria isolated from brinjal (*Solanum melongena L.*) plants identified as *Bacilluspolymyxa* when used as inoculum in field trials showed significant improvement growth parameters of treated plants over the uninoculated controls at all levels of fertilizer application. The isolate when multiplied and reinoculated showed competency in establishing themselves, fixing nitrogen and solubilizing phosphorus in the rhizosphere soil in field condition. Inoculation of brinjal both singly or in combination with *Azospirillum*, significantly enhanced the uptake of mineral and improved plant growth parameters. In a similar study Sukhada (1988, 1987) reported growth promotion under field conditions by bacteria isolated from the endorhizosphere of vegetables like tomato, okra and chillies. In okra and chillies these bacteria improved plant growth by increasing phosphate solubilization and releasing growth-promoting substances. These results demonstrated that nitrogen fixing and phosphate solubilizing rhizobacterial isolates could be effectively used to increase yield and save on the use of inorganic nitrogen and phosphatic fertilizers. In the present study plants, which received half dose of nitrogen and phosphorus and treated with *Bacillus polynya* showed a 47% increase in plant nitrogen and 23% increase in plant phosphorus over the uninoculated controls. Plants, which received full dose of nitrogen and phosphorus and treated with *Bacillus polymyxa* showed an improvement of only 12% and 17% in plant nitrogen and phosphorus over treated plants, which received half dose of nitrogen and phosphorus. An increase of 47% nitrogen were recorded in plots treated with *Bacilluspolymyxa* which is equivalent to 102 kgs of urea with an approximate cost of Rs. 737.00 if the cost of urea is Rs. 7.22 per kg and 23% phosphorus i.e., equivalent to 143 kg of super-phosphates with an approximate cost Rs. 2156.00 if the cost of superphosphates would be Rs 15 per Kg of phosphoric acid. Thus, use of *Bacilluspolymyxa* could almost reduce the cost of production of brinjal by almost half.

The application of *Bacillus polymyxa* along with full dose of nitrogen and phosphorus showed a 14% improvement in yield in treated brinjal plants that received half dose of nitrogen and phosphorus, 87% improvement over

untreated plants which received half dose of nitrogen and phosphorus and 100% improvement over untreated plants with zero dose of nitrogen and phosphorus. Plants treated with *Bacillus polymyxa* which received half dose of nitrogen and phosphorus showed 27% increase in shoot fresh weight and a 51% increase in plant height and 55% increase in photosynthetic efficiency and 87% improvement in yield over the uninoculated control which received the same level of fertilizers. Quimio and Coroza (1988) isolated and evaluated PGPR from the rhizosphere of tomatoes, white potatoes, sorghum, cowpea and corn. In corn they reported 24-43% increase in yield and 12-15% increase in dry weight. In sorghum, they reported an increase of up to 67% in yield and a dry weight increase of about 2-7%. In cowpea a yield increase of 28-42% and a dry weight increase of 13-48% was reported. Treated corn seedlings showed a significantly higher seedling weight, shoot length, root length and root weight. No increase in yield and growth parameters was observed when seeds of corn, rice, soybean and cotton were coated with *Rhizobacterium* cultures. *Zea mays* inoculation with *Azospirillum* by Kapulnik *et al.*, 1983 demonstrated that inoculated plants take up N P and K from mineral solutions significantly faster than the uninoculated controls.

Similarly, dry matter, N, P and K accumulated at greater rates in field-inoculated sorghum and wheat plants. According to Okon *et al.*, (1977) maximum nitrogen fixing activity was recorded during flowering. Linet *et al.*, (1983) obtained similar results when corn seeds were inoculated with *Azospirillum*. They reported significant increase in growth parameters and yield of treated plants over control. However, studies involving *Azotobacter* inoculation of seeds of cereals and vegetables in Soviet Union did not produce substantial and reproducible increase of yield for any plant (Mishustin 1969). Studies by Mehrotra and Lehri (1971) showed only small increase in yield. In almost all cases beneficial effects were observed only when nitrogenous fertilizers and FYM were applied.

Subba Rao (1983) summarized the beneficial effects of inoculation of seeds of cropplants with *Azotobacter*, particularly *Azotobacter chroococcum* and *Azospirillum brasilense*. They recorded an increase of 2 to 62 % depending upon the host plant, method of inoculation, locality and agroclimatic conditions. *Azospirillum* inoculation of sorghum resulted in a yield increase of 17 to 105 % in different regions. Pearl millet inoculation with sorghum gave a yield increase of 70% in some regions. Barea and Brown (1974) were able to obtain significantly high increase in plant growth and nitrogen content of both dicotyledonous and monocotyledonous plants incubated with *Azotobacter* (*Paspalum notatum*, *Lolium perenne* and *centrosema pubescens*). Inoculation with *Azotobacter* improved the growth of *Paspalum notatum* (Boddey and Dobereiner 1988; Fallik and Fischer, 1989).

The present study showed that the isolated strain of *Bacillus polymyxa* are effective inoculants, as they have the ability to establish themselves on the brinjal root surface in significant numbers and stimulate growth and increase yield. The isolated *Bacillus polymyxa* showed varied response at different growing seasons (Kharif and Rabi). It was observed that the inoculated plants were also quite resistant to infections. Extent of influence towards increase in yield and other growth parameters could be assessed if all the factors that biofertilization could be considered.

CONCLUSION

The beneficial effect of the isolated strain of *Bacillus polymyxa* used as inoculants for the cultivation of brinjal plants stimulated the growth and increased yield. Since inoculation with *B. polymyxa* caused a positive effect on plant development, further studies have to be taken to test if any growth promoting hormones were produced by this bacterial isolate which could be released to the plant environment.

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